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A review of gene mutations, conventional testing and novel approaches to cancer screening

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Abstract: Cancer is a genetic disease caused due to mutations in the tumor suppressor genes or oncogenes involved in the cell cycle regulation. It may include mutations that may be inherited or acquired during one's lifetime and affect single gene or multiple genes, chromosomes and their protein expression patterns, ultimately leading to a loss of control over the cell cycle and culminating in uncontrolled cell growth. With the tremendous increase in global cancer burden and early detection being the key to cure, it has become imperative that the genes be studied and new genetic and biochemical testing techniques be utilized. In this review, we have looked at various mutations involved in common cancer-causing genes, their role under normal physiological conditions, mechanisms of mutation and their occurrence in different types of cancers. Also, the review focuses on conventional and novel approaches for genetic and biochemical testing, the techniques used and their advantages and limitations.

Introduction

Cancer is a genetic disease caused due to mutations in single or multiple genes or chromosomes, leading to the loss of control over the cell cycle and uncontrolled growth of cells (Milne et al., 2011). A gene is the basic physical and functional unit of heredity. Genes are present on 46 chromosomes which are divided into sets of two, each containing 23 chromosomes. They perform vital cell functions, including how fast a cell grows, how often it divides and how long the cell lives. They also code for proteins that perform specific bodily functions and act as messengers for the cells. Genes can undergo changes known as mutations which may lead to the production of non-functional proteins. Others may cause the cells to evade the normal growth controls leading to uncontrolled growth and, ultimately, cancer. Genetic changes that promote cancer may be inherited from parents, known as germ line changes acquired during one's lifetime aromatic changes (Balakrishnan et al., 2007). In normal cells, tumour suppressor genes work to prevent excessive and inappropriate cell growth. Damage to them may lead to cancer (Cox and Chen et al., 1994). Also present are proto-oncogenes that may undergo mutations to convert to oncogenes and cause cancer (Slamon, 1987). Specific genes are involved in the development of specific types of cancers. With the growing burden of cancer incidence and mortality worldwide, there is a need for advanced cancer testing to predict people who may be susceptible to cancer or detect cancer at an early stage to improve survival (Loud et al., 2017). With the advances in research, various types of testing have been done to detect cancer (Caiand Liu, 2019). This review provides an overview of some of the most common gene mutations found in different cancers (Figure 1). The mutation mechanism, role and occurrence of eleven representative genes related to cancer are discussed in detail. The latter part describes cancer

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screening tests, including genetic, biochemical, and AI-based tests.

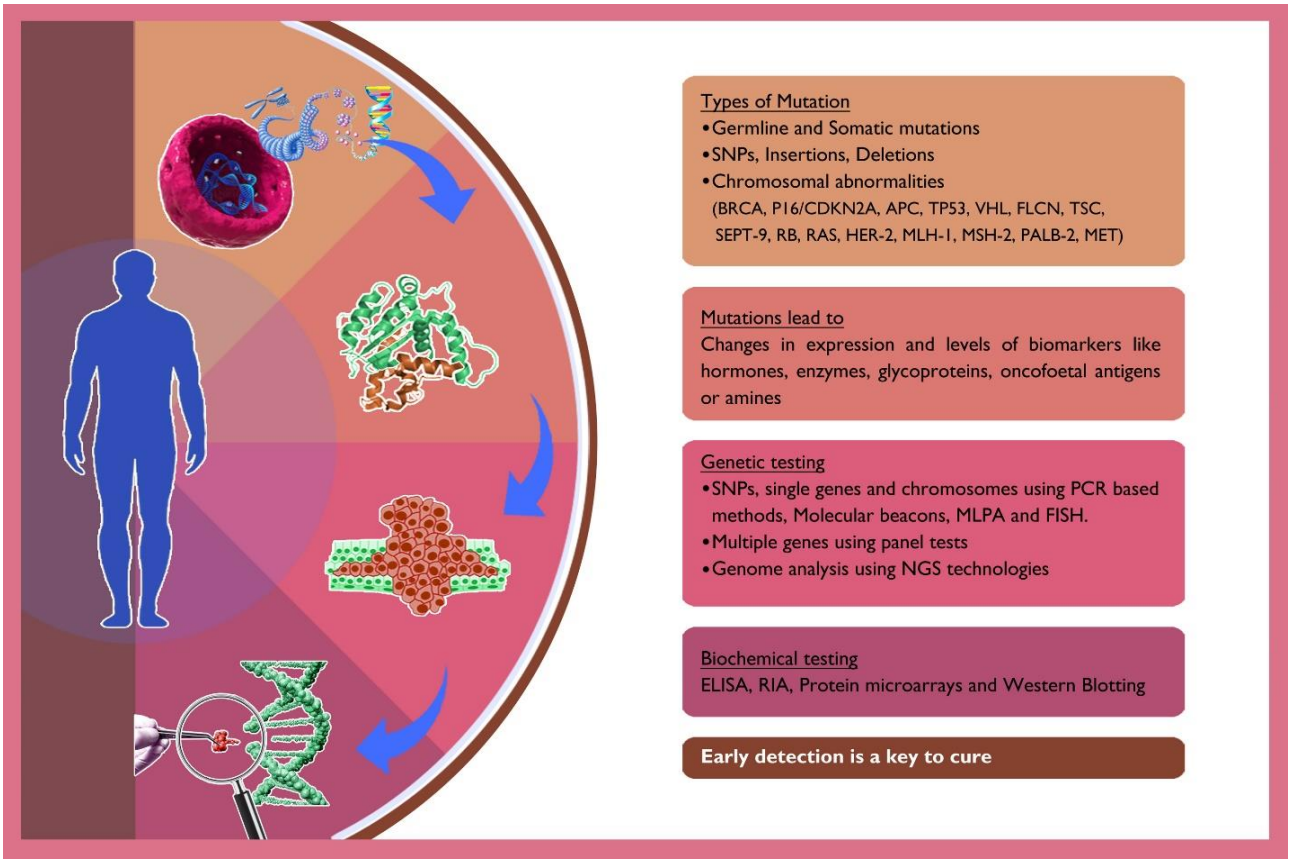

Figure 1. Various mutation types, mutation causes and tests are shown

Types of mutations

Mutation is a permanent change in the nucleotide sequence of deoxyribose nucleic acid (DNA) that leads to a change in the structure of a gene. It can lead to changes in the structure of proteins or a decrease or loss in their expression. Table 1 summarizes different types of mutations based on varied parameters.

1. Mutations can be classified based on whether they are inherited from parents or occur during the lifetime of an individual as:

Germline mutations

Here the mutations are inherited from the parents to the offspring. This is because te mutations are carried in the germ cells like eggs and sperm(Balakrishnan et al., 2007).

Somatic mutations

Here the mutations are acquired during an individual's lifetime to exposure to certain mutagens (Balakrishnan et al., 2007).

2. Mutations can also be classified based on whether they affect a gene or a chromosome:

Gene mutations

These mutations occur on a small scale, generally affecting a single gene. They are caused due to mutagen-induced DNA damage, errors in DNA synthesis or faulty DNA repair mechanisms. They include:

Point mutation

These are the mutations wherein there is a change in only a single nucleotide base pair. It can occur in the following three ways:

Missense mutation

A mutation in which one amino acid is replaced by another amino acid (Molly Campbell, 2020).

Nonsense mutation

The translation is prematurely terminated by mutations that replace amino acid codons with stop codons (Molly Campbell, 2020).

Frameshift mutations

These mutations cause changes in the reading frame that leads to the incorporation of an unrelated amino acid (Hatfield et al., 1990).

Single nucleotide polymorphs (SNPs) are point mutations involving single nucleotide variation at a specific location in the genome (Kim and Misra, 2007). The other two types of gene mutations found are:

Insertions

Involves the addition of one or more nucleotide base pairs to the DNA sequence.

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Deletions

Involves the loss of one or more nucleotide base pairs from the DNA sequence (Armour et al., 2002; Loeb and Loeb, 2000).

Mutations can also be classified based on the number of alleles affected:

Heterozygous mutation

Here only one of the alleles of the pair is mutated.

Table 1. Classification of mutations based on various parameters

Basis of classification	Name of the mutation	Types	Definition	References
Mechanism of obtaining the mutation	Germline	NA	Inherited from parents to offspring	Balakrishnan et al., 2007
	Somatic	NA	Acquired during lifetime	
Part of the genetic material affected	Gene	Point mutations (Missense, Nonsense, Frameshift mutations)	Changes in single nucleotide base pair	Armour et al., 2002; Hatfield et al., 1990; Kim and Misra, 2007; Loeb and Loeb, 2000; Mahdieh and Rabbani, 2013
		Insertions	Addition of one or more nucleotide base pairs	
		Deletions	Loss of one or more nucleotide base pairs	
	Chromosome	Translocation	Interchange of genetic material between nonhomologous chromosomes	
		Deletion	A region of the chromosome is deleted.	
		Duplication	A region of the chromosome is duplicated.	
		Aneuploidy	An abnormal number of chromosomes	
Number of alleles affected	Homozygous	NA	Only one of the alleles of the pair is mutated	van Boxtel et al., 2011
	Heterozygous	NA	Both alleles are mutated	

Chromosomal changes

These are large-scale changes and affect multiple genes. They are caused by chromosome pairing errors and crossing-over errors that occur during meiosis. They mainly include:

Translocation

Involves interchange of genetic material between non-homologous chromosomes

Deletion

A region of the chromosome is deleted.

Duplication

A region of the chromosome is duplicated.

Aneuploidy

Involves an abnormal number of chromosomes (Loeb and Loeb, 2000; Mahdieh and Rabbani, 2013).

Homozygous mutation

Here both alleles undergo mutation (van Boxtel et al., 2011).

Tumour suppressor genes and oncogenes

Tumour suppressor genes are genes involved in preventing cancer. Loss of function mutations in both alleles causes the inactivation of these genes leading to uncontrolled cell growth. The mutations that occur may be either two somatic mutations wherein both the alleles get mutated or a germline mutation in which one of the alleles of the pair is already mutated and inherited from a parent, followed by a mutation in the other normal allele which leads to the loss of function (Hooper, 1998).

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Table 2. Genes, along with their mutation type and involvement in causing a particular type of cancer

Name of the gene	Type of the gene	Mutation type	Type of cancer	References
BRCA	Tumour suppressor	Germline mutations	Breast, ovarian cancer, colon cancer, prostate cancer pancreatic cancer.	Greenberg, 2006; Mersch et al., 2015; Tapia et al., 2008; Venkitaraman, 2009
P16	Tumour suppressor	Homozygous deletions, point mutation or methylation of the p16 promoter.	Oesophageal cancer, lung cancer and laryngeal cancers, gastric cancers, and skin cancers.	Caldas et al., 1994; Foulkes et al., 1997; Helgadottir et al., 2014; Igaki et al., 1994; Liggett Jr. and Sidransky, 1998; Puig-Butille et al., 2014; Rayess et al., 2012
APC	Tumour suppressor	Germline mutations have alterations in the seven CpA and five CpG sites or somatic mutations involve alterations in the five CpG and three CpA regions. These lead to truncation products either due to point mutations or frameshifts.	Colorectal cancer	Fodde, 2002; Markowitz and Bertagnolli, 2009; Miyoshi et al., 1992; Spirio et al., 1993
TP53	Tumour suppressor	Inactivation of the gene through single base substitution and loss of alleles, germline mutation and increase in the number of polymorphs	Hepatocellular carcinoma, head and neck cancers, lung cancer and melanomas.	Boyle et al., 1993; Denissenko et al., 1996; Hollstein et al., 1991; Oda et al., 1992; Olivier et al., 2010; Ziegler et al., 1993
VHL	Tumour suppressor	Germline or somatic mutations. Somatic mutations mainly include deletions, missense and indel or splice site mutations.	Sporadic renal carcinoma	Gnarra et al., 1994; Maher et al., 2011; Nordstrom-O'Brien et al., 2010

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FLCN	Tumour suppressor	Germline which is mostly caused by frameshift mutations or nonsense mutations that lead to protein truncation.	Renal carcinoma	Menko et al., 2009
TSC1/ TSC2	Tumour suppressor	Germline or somatic mutations.	Brain, kidney, skin, heart and lung cancer.	Huang and Manning, 2008; Inoki et al., 2006; Sampson and Harris, 1994
SEPT9	Tumour suppressor	Methylation at CpG islands.	Colorectal cancer	Connolly et al., 2011; Molnar et al., 2015; Tokhtaeva et al., 2015; Toth et al., 2011; Wasserkort et al., 2013
Rb	Tumour suppressor	Large or small deletions, nonsense mutations, splice mutations or missense mutations.	Small cell lung cancer, non-small cell lung cancer, pancreatic cancer, breast cancer, glioblastoma-multiforme, mantle cell lymphoma and intraocular malignancies in children.	Ali et al., 2010; Murphreeand Benedict, 1984; Shao and Robbins, 1995; Sherrand McCormick, 2002
RAS	Proto-oncogene	Point mutations mainly convert from glycine (GGT) to aspartic acid, valine (GTT) or arginine (CGT).	Pancreatic carcinoma, extrahepatic bile duct carcinoma, gall bladder, and colorectal and lung carcinoma.	Hezel et al., 2014; Jancik et al., 2010; Pramanik et al., 2011; Samatar and Poulikakos, 2014; Tada et al., 1991
HER	Proto-oncogene	Single nucleotide changes like the change from Val to Glu at position 664. Gln and Asp also activate neu at this position.	Breast cancer, gastric cancer, ovarian, stomach cancer, adeno-carcinoma of the lungs and aggressive uterine cancers.	Hynes and Stern, 1994; Mazieres et al., 2013; Menard et al., 2003; Mitri et al., 2012; Santin et al., 2002; Tai et al., 2010

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MLH-1	Tumour suppressor	Germline or somatic mutations include frameshift mutations, nonsense mutations, missense mutations or splice site mutations.	Colorectal cancer, stomach cancer, oesophageal cancer, head and neck squamous cell carcinoma and non-small cell lung cancer.	Cunningham et al., 1998; Kunkel and Erie, 2005; Seng et al., 2008; Tawfik et al., 2011; Truninger et al., 2005; Uehara et al., 2005
MSH-2	Tumour suppressor	Germline or somatic mutations which include frameshift mutations, nonsense mutations, missense mutations or splice site mutations.	Colorectal cancer	de Wind et al., 1995; Fishel et al., 1993; Wagner et al., 2003
PALB-2	Tumour suppressor	Truncated mutations	Pancreatic cancer, breast cancer	Antoniou et al., 2014; Jones et al., 2009; Xia et al., 2006
MET	Proto-oncogene	Germline or somatic mutations which mostly include missense mutations	Stomach, lung and colorectal cancer	Kuniyasu et al., 1992; Naldini et al., 1991; L. Schmidt et al., 1997; Zhang et al., 2018

Oncogenes are genes that encode proteins required to regulate cell cycle proliferation and apoptosis. Mutations in these genes lead to their activation leading to cancer. Table 2 describes genes with their mutation types and prevalence in various types of cancers. Activation of an oncogene is necessary but requires alterations in other genes for the development of cancer (Croce, 2008). Following are the various genes involved in cancer.

Breast cancer (*BRCA*) gene

Type of gene and mutation mechanism

The *BRCA* genes, namely Breast cancer type 1 susceptibility protein (*BRCA1)* and Breast cancer type 2 susceptibility proteins *(BRCA2)*, encode for breast cancer type 1 susceptibility protein and breast cancer type 2 susceptibility protein, respectively and help maintain the stability of chromosomes during replication and thus act as tumour suppressor genes. Since humans have a diploid genome, each cell has two gene copies. *BRCA* gene mutation is an inherited type of mutation in which one of the alleles is mutated, thus making the person heterozygous for the mutation.

The loss of heterozygosity occurs if the functional copy is also harmed, thus forcing the cell to use alternate erroneous mechanisms for DNA repair leading to cancerous transformations (Greenberg, 2006). Many mutations have been identified, including point mutations or large segment mutations involving the deletion or duplication of one or several exons of the *BRCA* gene. Hyper-methylation of the *BRCA* gene promoter has also been identified in certain cancers (Tapia et al., 2008).

Functions and Role in Cancer

In normal cells, during replication, the DNA polymerase stalls at lesions caused on the template strand due to oxidative damage to the nitrogenous base pairs. This causes an arrest of the replication fork, preventing further replication. DNA at this site is cleaved to generate double-stranded DNA breaks (DSBs). These DSBs are repaired in humans via two major pathways. First, non-homologous end joining (NHEJ) ligates the ends of the strands irrespective of their homology leading to error-prone and mutagenic repair. The other pathway is the repair of the damaged DNA strand using an intact,

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homologous template as a sequence known as error-free homologous DNA recombination (HR). *BRCA1* and *BRCA2* are essential for efficient HR. Cells in which the BRCA genes are mutated are deficient in HR, but NHEJ can still lead to mutagenic changes and, thus, cancer (Venkitaraman, 2009).

Occurrence

Mutations in BRCA1 and BRCA2 genes increase a person's susceptibility toward breast and ovarian cancer and certain other cancers like colon, pancreatic, and prostate (Mersch et al., 2015).

P16 cyclin dependent kinase inhibitor 2a(*P16/CDKN2A*) gene

Type of gene and mutation mechanism

P16/CDKN2A is a tumour suppressor protein encoded by the *CDKN2A* gene. It regulates the cell cycle by reducing the rate at which cells progress from G1 to the S phase, thus acting as a tumour suppressor and preventing uncontrolled cell growth (Foulkes et al., 1997). Mutations in the *CDKN2A* gene result in losing control over the cell cycle leading to cancer. They may be homozygous deletions, point mutation or methylation of the p16 promoter. Methylation of 5' regulatory regions or discrete regions of CG dinucleotides called CpG islands is an important mechanism of transcriptional repression. It may lead to complete transcriptional blockage. These CpG islands are found to be unmethylated in normal cells (Liggett Jr. andSidransky, 1998).

Functions and Role in Cancer

CDK binds to cyclin D and causes phosphorylation of retinoblastoma protein (pRB). On phosphorylation,pRB enters the nucleus and causes transcription of proteins required for the cell transition from G1 to the S phase. p16 binds to CDK and prevents its interaction with cyclin D thus arresting cell growth. Mutation of p16 leads to cancer because of dysregulation of the cell cycle (Rayess et al., 2012).

Occurrence

Pancreatic adenocarcinoma is associated with *CDKN2A* mutation (Caldas et al., 1994). Germline mutations in *CDKN2A* cause cancers like oesophageal, lung, and laryngeal cancers. Tobacco smoking increases the susceptibility of the carrier to such cancers(Helgadottir et al., 2014). Oesophageal and gastric cancers may be caused by homozygous deletion (Igaki et al., 1994). Skin cancer risk may increase to germline mutations (Puig-Butille et al., 2014).

Adenomatous Polyposis Coli (*APC*)gene

Type of gene and mutation mechanism

APC is a protein encoded by the *APC gene* (Aghabozorgi et al., 2019). It acts as a tumour suppressor gene. It regulates various cell processes that decide whether a cell would become cancerous. Mutations may occur in germline mutations with alterations in the seven CpA and five CpG sites or somatic mutations involving alterations in the five CpG and three CpA regions(Miyoshi et al., 1992). These lead to truncation products either due to point mutations or frame shifts (Spirio et al., 1993).

Functions and Role in Cancer

APC protein acts by forming a destruction complex with axin and glycogen synthase kinase (GSK). This complex then binds to β-catenin, which is phosphorylated and degraded by cellular proteasomes. β-catenin is a protein involved in cell proliferation by activation mitosis. Since APC is involved in targeting β-catenin for destruction, its translocation into the nucleus is prevented and leads to inhibiting action as a transcription factor for proliferation genes (Fodde, 2002).

Occurrence

APC gene mutation is the typical type of mutation resulting in colon cancer (Markowitz and Bertagnolli, 2009).

Tumour protein 53 (*TP53*) gene

Type of gene and mutation mechanism

TP53 or *p53gene*, also known as the 'guardian of the genome' is a tumour suppressor gene that encodes for a tumour protein p53. It plays an important role in tumour suppression (Rivlin et al., 2011).

In the absence of cellular stress, p53 is present in cells in its latent form. When the cells are exposed to stressors like direct DNA damage, chromosomal aberrations, telomere shortening and activation of oncogenes, it leads to the activation of p53 (Mantovani et al., 2019). The abundance of p53 in the cells increases due to its activation. p53 then binds to the response elements and activates transcription genes. The proteins encoded by these genes help to repair the damaged DNA. When the damage is irreparable, the cell is directed to undergo apoptosis, thus preventing tumorigenesis. Mutations in p53 thus lead to the accumulation of unrepaired DNA leading to cancer (Rivlin et al., 2011).

Functions and Role in Cancer

The mutations in *TP53* are most frequently found in almost all types of DNA damage. Genetic variations in

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TP53 contributes to cancer development in three ways (Olivier et al., 2010).

The first is somatic mutations that cause the inactivation of the gene through single base substitution and loss of alleles.

Second is the germline mutation in which the mutated allele is inherited from either of the parents making the person susceptible to an array of early onset cancers like breast carcinoma, sarcomas, brain tumour and adrenal cortical carcinomas defining the Li-Fraumeni Syndrome(LFS)(Olivier et al., 2010).

The third mechanism involves enhanced polymorphism in *TP53* since *TP53* is highly polymorphic in coding and non-coding regions. An increase in this polymorphism has been attributed to the development of cancers. Though most tumour suppressor genes undergo frameshift or nonsense mutations, TP53 mutations are mostly missense and cause variations in the single amino acid at many different locations (Hollstein et al., 1991).

Occurrence

TP53 mutations are commonly found in hepatocellular carcinoma, neck and head cancers, and lung cancers (Boyle et al., 1993; Denissenko et al., 1996; Ziegler et al., 1993; Oda et al., 1992).

Von hippellandau (*VHL*) gene

Type of gene and mutation mechanism

VHL is a tumour suppressor gene that encodes for VHL protein. More than 1500 germline and somatic mutations have been identified in the *VHL* gene, leading to sporadic tumour development. Germline mutations are caused when a mutated allele is acquired from either of the parents. Somatic mutations have also been found wherein deletions contributed to 30-40% of cases. The remaining 60-70% of cases were attributed to the truncation of VHL proteins by missense, indel or splice site mutations (Nordstrom-O'Brien et al., 2010).

Function and role in cancer

*VHL*gene is involved in regulating hypoxia-inducible factor 1 alpha (HIF-1α). At normal cellular oxygen levels, VHL binds to HIF-1α and ubiquitinates it causing it to undergo proteasomal degradation. But in conditions of hypoxia or when the gene is mutated, it is unable to bindHIF-1α. This prevents its degradation and leads to the transcription of a multitude of genes, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), erythropoietin and many others leading to uncontrolled cell growth (Maher et al., 2011).

Occurrence

VHL mutation is commonly found in sporadic renal carcinoma (Gnarra et al., 1994).

Folliculin (*FLCN*) gene

Type of gene and mutation mechanism

FLCN gene, also known as Birt-Hogg-Dupe (BHD), encodes for a protein called folliculin. Its exact function is unknown, but research suggests it acts as a tumour suppressor gene. The mutations observed in this gene are mainly germ lines, primarily caused by frameshift mutations or nonsense mutations that lead to protein truncation. A small percentage of mutations are splice site alterations. Loss of heterozygosity is mainly responsible for cancer development (Schmidt et al., 2018).

Function and Role in Cancer

FLCN regulates the mammalian target of the rapamycin (mTOR) pathway, which may play a role in tumorigenesis though the exact function is unclear.

Occurrence

FLCN gene mutation has been found in renal carcinomas and has been shown to increase the risk of renal carcinoma up to seven times when compared to individuals who do not carry this mutation (Menko et al., 2009).

Tuberous sclerosis (*TSC*) genes

Type of gene and mutation mechanism

Tuberous sclerosis proteins, also known as TSC1 (hamartin) and TSC2 (tuberin), which are encoded by the *TSC1* and *TSC2* genes, respectively, form a complex which acts as a tumour suppressor (Inoki et al., 2006; Ghosh et al., 2006). The mutations in the gene may be somatic or germ-line and follow the two-hit hypothesis in which the first hit, which is the first mutation, leads to heterozygosity. The second hit, the second mutation, leads to loss of heterozygosity and thus cancer (Sampson and Harris, 1994).

Function and Role in Cancer

The TSC1 and TSC2 form a complex in which TSC2 has a catalytic subunit having guanosine triphosphatase (GTPase) activity towards Ras homolog enriched in brain (RHEB), a Ras family GTPase. RHEB causes activation of mTOR and thus leads to the formation of two complexes Transducer of regulated CREB activity (TORC). The first is TORC1 is sensitive to rapamycin and causes phosphorylation of ribosomal S6 kinase 1 (S6K) and eukaryotic initiation factor 4E binding protein 1 (4EBP1), which regulate translation. The second complex that is activated is TORC2 which causes phosphorylation and activation of protein kinase B (PKB), which is involved in regulating many cellular processes. Mutation in the *TSC* genes leads to loss of

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control over protein synthesis and cellular growth leading to cancer (Inoki et al., 2006; Ghosh et al., 2006).

Occurrence

The mutations in this gene lead to the development of autosomal dominant tumour syndrome, leading to tumours affecting the brain, kidney, skin, heart and lungs (Huang and Manning, 2008).

Septin-9 (*SEPT9*) gene

Type of gene and mutation mechanism

Septin-9 is a protein encoded by the *SEPT9* gene. It regulates cellular growth and exocytosis (Tokhtaeva et al., 2015). Mutations in this gene lead to the development of various types of cancers. The mutations mainly include methylation at the various positions of the CpG islands (Wasserkort et al., 2013).

Function and Role in Cancer

SEPT9 is a highly conserved family of septin genes coding for GTP-binding proteins. These multi-domain proteins assemble into complexes and form filamentous structures which comprise a part of the cytoskeleton. These proteins play important roles in many cellular processes by providing rigidity to the cell membrane, serving as scaffolds to recruit proteins to specific subcellular locales and creating membrane diffusion barriers to establish discrete cellular domains (Wasserkort et al., 2013).

Occurrence

The v1 region is hypermethylated in breast, ovarian and prostate cancer cases. *SEPT9* amplification occurs at the DNA level during human breast carcinogenesis and results in an overall increase in SEPT9 mRNA and protein levels(Connolly et al., 2011). In the case of colon cancer, the v2 region of the promoter is found to be hypermethylated; in the case of breast cancer, v3 region is hypermethylated (Connolly et al., 2011; Toth et al., 2011). A new test known as Epiprocolon has been developed to detect septin-9 methylation in the case of colorectal carcinoma (Molnar et al., 2015).

9. Retinoblastoma (*RB*)gene

Type of gene and mutation mechanism

The *RB* gene is a tumour suppressor gene that is dysfunctional in the case of many cancers, such as bladder cancer, breast cancer and lung cancer (Murphree et al., 1984; Du et al., 2009; Witkiewicz et al., 2014). The main function of the gene is to prevent excessive cell division. It does this by arresting cell progression until it is ready to divide. The *RB* gene is inactivated following phosphorylation once the cell is ready to divide and thus allows cell cycle progression (Shao and Robbins, 1995). The mutations in this gene also follow the two-hit hypothesis. Mutations of both alleles are required for cancerous growth to be initiated. The mutations involved may be large or small deletions, nonsense, splice, or missense mutations. Over 900 mutations at different sites have been identified (Ali et al., 2010).

Function and Role in Cancer

RB gene mainly functions in cancer prevention by preventing the progression of the cell from the G1 phase to the S phase. It binds to and inhibits the transcription factors of the E2F family. It is composed of E2F proteins and a dimerization partner (DP) protein. When activated, the E2F-DP complex causes the progression of the cell from the G1 to the S phase. As the RB gene inactivates it, the cell cannot proceed to the S phase and is thus arrested in the G1 phase, thus preventing cancer. Thus, *RB* gene mutation leads to a loss of control over the cell cycle leading to cancerous growth (Sherrand McCormick, 2002).

Occurrence

RB gene mutation is responsible for developing small cell lung cancer, non-small cell lung cancer, pancreatic cancer, breast cancer, glioblastoma multi-forme, mantle cell lymphoma and intraocular malignancies in children (Ali et al., 2010; Sherrand McCormick, 2002).

Ras genes

Type of gene and mutation mechanism

The RAS family consists mainly of three genes: *KRAS*, *HRAS* and *NRAS*. It is a proto-oncogene and mutations in these genes are responsible for various types of cancers. They mainly include point mutations at codons 12, 13 or 61, which convert the proto-oncogene to an oncogene. The types of mutations found are mainly conversion from glycine (GGT) to aspartic acid (GAT), valine (GTT) or arginine (CGT)(Tada and Omata, 1991; Rajasekharan and Raman, 2013).

Function and Role in Cancer

The *RAS* gene is involved in the signalling pathway known as Ras-Raf-mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK). Once activated, the RAS gene increases the RAF gene's phosphorylation. The Raf kinases further cause the phosphorylation of MAPK 1 and MAPK 2. Once phosphorylated, these MAPKs cause phosphorylation and activation of ERK. Activated ERK then translocate to the nucleus and regulates various transcription factors that ultimately lead to changes in gene expression (Samatar and Poulikakos, 2014).

Occurrence

It is found to be mutated in the cases of pancreatic carcinoma, extrahepatic bile duct carcinoma, gall bladder

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carcinoma, colorectal carcinoma and lung carcinoma. In almost 90% of pancreatic cancers, the *KRAS* gene is found to be mutated (Tada et al., 1991; Hezel et al., 2014; Pramanik et al., 2011; Jancik et al., 2010).

Human epidermal growth factor receptor-2 (*HER2*) gene

Type of gene and mutation mechanism

HER2 gene, also known as *neu* or erythroblastic oncogene B (*ERBB*), is a proto-oncogene that is found to be mutated in a large number of human cancers such as breast cancer and gastric cancer (Yano et al., 2004; Burstein, 2005). It is closely related to the ERBB gene that encodes for the Epidermal Growth Factor Receptor (EGFR). Mutations involved are single nucleotide changes like the change from Val to Glu at position 664. Gln and Asp also activate neu at this position. Overexpression is mainly responsible for tumorigenesis (Hynes and Stern, 1994).

Function and Role in Cancer

HER2 proteins are trans-membrane proteins that consist of intracellular and extracellular domains. The ligand binds to the extracellular domain and causes conformational changes that lead to receptor dimerization. This further causes the activation of signalling pathways that are involved in cell proliferation and apoptosis. Thus, overexpression of *HER2* leads to loss of control over the cell cycle and, thus, cancer (Menard et al., 2003).

Occurrence

HER2 mutations are responsible for cancers of the ovary, and stomach, aggressive uterine cancers, and adenocarcinoma of the lungs and breast (Mazieres et al., 2013; Mitri et al., 2012; Santin et al., 2002; Tai et al., 2010).

Mutl Homolog-1 (*MLH1*) gene

Type of gene and mutation mechanism

The *MLH1* gene is a gene that codes for the MLH1 protein. It is one of the components of the system of seven proteins called DNA mismatch repair proteins that work in sequential steps to repair the mismatched DNA. Unfortunately, the mutations lead to an inability to repair DNA, thus causing cancer. Mutations may be germline or somatic, including frameshift mutations, nonsense mutations, missense mutations or splice site mutations wherein the promoter is hyper-methylated in most cases (Cunningham et al., 1998).

Function and Role in Cancer

During DNA replication, errors occur wherein the bases may be paired wrongly, or there may be an addition or deletion of small sequences from one of the strands that lead to mismatching with the other strand. These errors must be identified and repaired. If they remain unrepaired, they may lead to microsatellite instability and mutations that are responsible for tumorigenesis. Since *MLH1* gene is involved in the DNA repair process, its mutation causes a faulty DNA repair mechanism leading to cancer (Kunkel and Erie, 2005).

Occurrence

Mutated *MLH1* gene is found to be one of the major causes of colorectal cancer (Truninger et al., 2005). It is also responsible for cancers like oesophageal cancer, head and neck squamous cell carcinoma and non-small cell lung cancer (Seng et al., 2008; Tawfik et al., 2011; Uehara et al., 2005).

MutsHomolog-2 (*MSH2*)gene

Type of gene and mutation mechanism

MSH2 gene has a similar function to that of the *MLH1* gene. It is also a DNA repair gene that codes for the MSH-2 protein. The mutations that occur are also similar to that in the *MLH1* gene (Wagner et al., 2003).

Function and Role in Cancer

MSH2 and MSH6 form a heterodimer that first recognizes the DNA mismatch. Also, MSH2 and MSH3 can form a heterodimer and start the process of mismatch detection. Once the MSH2-MSH6 heterodimer is formed, it recruits a second heterodimer of MLH1 and mismatch repair system component-2 (PMS2). Either PMS3 or MLH3 can substitute for PMS2. This protein complex formed between the 2 sets of heterodimers enables the initiation of repair of the mismatch defect. Mutations cause a faulty DNA repair mechanism, thus leading to cancer (de Wind et al., 1995).

Occurrence

Mutations in *MSH2* gene are mainly associated with colorectal cancer, also known as Lynch syndrome (Fishel et al., 1993).

Partner and localizer of brca2 (*PALB2*) gene

Type of gene and mutation mechanism

PALB2 gene codes for a protein that binds to the *BRCA* gene and is involved in the DNA repair pathway. Mutations mainly found are truncated mutations that are inherited from either of the parents. Truncated mutations result in a premature stop codon or a nonsense codon in the mRNA that has been transcribed, leading to the formation of a truncated and incompletely formed protein that is usually non-functional (Jones et al., 2009).

Function and Role in Cancer

The protein PALB2 binds to *BRCA* gene and colocalizes with the BRCA gene in the nuclear foci. It aids in localisation and helps maintain the stability of

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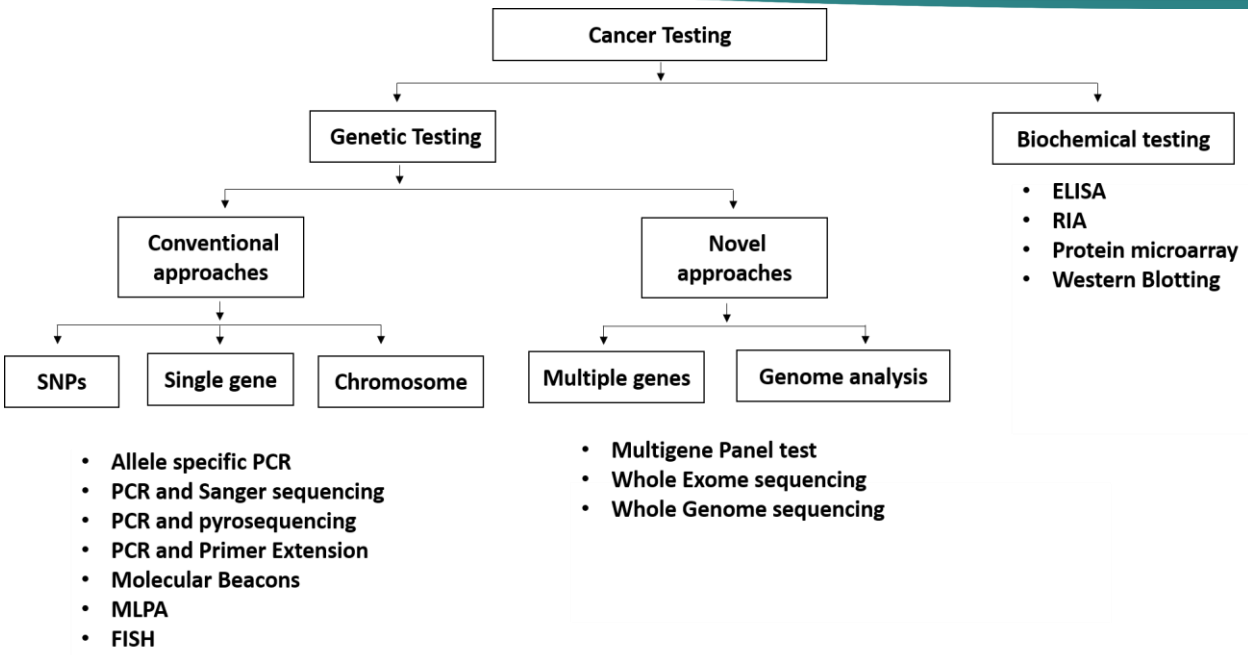

Figure 2. Classification of cancer testing

nuclear structures like chromatin and nuclear matrix. It is also involved in recombinational repair and checkpoint functions. Thus, *PALB2* is a key regulator of the activities of the *BRCA* gene and ensures that the *BRCA* gene can carry out its tumour-suppressing function efficiently. Thus, mutations in *PALB2*gene lead to faulty repair and disruption of the activity of *BRCA*, leading to cancer(Xia et al., 2006).

Occurrence

It is a key gene in pancreatic cancer (Jones et al., 2009). Also, mutations in the *PALB2* gene increase a person's susceptibility to breast cancer (Antoniou et al., 2014).

Met proto-oncogene, receptor tyrosine kinase (*MET*) gene

Type of gene and mutation mechanism

MET gene is a proto-oncogene that codes for a protein called c-MET. The protein possesses tyrosine kinase activity (Naldini et al., 1991). The mutations involved may be germline or somatic, with most of the mutations being missense mutations. These missense mutations cause a single nucleotide change, resulting in a codon that codes for a different amino acid than regular (Schmidt et al., 1997).

Function and Role in Cancer

MET pathway plays a role in the development of cancer through different pathways. They cause the activation of key oncogenic regulators like Ras, phosphoinositide-3-phosphate (P13K), beta-catenin and signal transducer and activator of transcription-3 (STAT-3). They are also involved in angiogenesis. Another the pathway is the production of metallo-proteinases that cause cell dissociation and lead to metastasis (Zhang et al., 2018).

Occurrence

MET gene mutation is majorly responsible for papillary renal carcinoma (Schmidt et al., 1997). It is found to be amplified in other types of cancers like scirrhous type stomach cancer, lung cancer and colorectal cancer (di Renzo et al., 1995; Kuniyasu et al., 1992; Lutterbach et al., 2007).

Cancer testing

Cancer testing involves testing for various genetic, biochemical or genomic changes. Conventional genetic testing involves testing for changes in chromosomes, genes and SNPs using techniques like Fluorescent in-situ hybridization (FISH), Multiplex ligation-dependent probe amplification (MLPA), Allele-specific PCR, PCR and Sanger sequencing, PCR and pyro-sequencing, PCR and Single-Base Extension and Molecular beacons. Novel approaches to genetic cancer testing dwell on Next generation sequencing (NGS) for multi-gene and genomic analysis. Multi-gene panel testing tests many genes at a time, while genomic testing includes whole exome and whole genome sequencing. Apart from genetic testing, cancer testing also utilizes biochemical testing, which analyses various protein markers through techniques like Radioimmunoassay (RIA), Enzyme-linked immune-sorbent assay (ELISA), protein microarrays and western blotting. Figure 2 depicts the detailed classification of cancer testing.

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Table 3. Summary of different approaches and techniques used in cancer testing, the type of mutations they detect and their advantages and disadvantages.

Type of Testing	Name of Technique	Changes detected	Advantages	Disadvantages	References
Conventional genetic testing	Allele-specific PCR	SNPs	1) Low cost 2) Fast technique 3) Simple procedure 4) Cost effective	1) The single tube reaction may add to the cost of the reaction due to the requirement of multiple fluorescent probes to detect different products	Gaudet et al., 2009
	PCR and Sanger sequencing	SNPs and small variants	1) Efficient 2) Has good sensitivity	1) Time-consuming 2) Risky due to the use of radioisotopes for labelling 3) Poor speed and sequencing quality 4) Labour intensive	Ari and Arikan, 2016; Katsanis and Katsanis, 2013; Wang et al., 2019
	PCR and pyro-sequencing	SNPs	1) Rapid 2) Accurate 3) Easy to accomplish	1) High cost 2) Enables only short reads of nucleotides (up to 70) 3) Annealing specificity of the primer may be affected due to the temperature at which it is performed (28^{o}C) 4) Difficulty sequencing GC-rich templates 5) High background signal	Ari and Arikan, 2016; Clarke, 2005; Fakhrai-Rad et al., 2002
	PCR and Primer Extension	SNPs	1) Highly accurate 2) High specificity 3) Robust 3) Not affected by small changes in reaction conditions 4) Useful to differentiate homozygous and heterozygous genotypes	1) Requires removal of PCR primers and dNTPs before detection of the single nucleotide primer extension 2) Though theoretically, it has been found to be accurate and specific, some discrepancies have been observed practically	Sobrino et al., 2005; Syvanen, 1999

	Molecular Beacons	SNPs	1) Simple technique 2) Robust 3) Multiple targets in the same solution can be detected by using different fluorescent probes 4) High specificity 5) Good selectivity 6) High signal-to-background ratio	1) High background when auto-fluorescent cells are present 2) False positive results	Marras et al., 2003
	MLPA	Chromo-somes	1) Fast, precise and reliable technique 2) Inexpensive technique 3) Accurate 4) Sensitive 5) Not labour intensive 6) Suitable when a large number of samples are to be analyzed	1) False positive results 2) Very low amount of DNA (< 20ng) can affect the MLPA peak 4) Use of reference samples without proper buffering can lead to abnormal peaks	Homig-Holzel and Savola, 2012
	FISH	Chromo-somes	1) Easy to use 2) Fast analysis 3) Good sensitivity 4) Hundreds of tests can be performed on the same tissue sample	1) Affected by the presence of artefacts which makes interpretation difficult 2) Quantification is difficult 3) Low amplification efficiency 4) Poor reproducibility	Eastmond et al., 1995; Jensen, 2014; Katsanis and Katsanis, 2013
Novel genetic testing	Multigene panel testing	Multiple genes	1) Cheap 2) Fast 3) Has better efficiency than the other techniques 4) Can analyze changes in	1) Higher the number of genes sequenced higher the variance of uncertain significance (VUS) 2) Requires skilled genetic counsellor to avoid	Kurian and Ford, 2015

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			multiple genes at a time, leading to improved risk assessment, early detection and prevention.	misinterpretation 3) Complicated when genes to be analysed are less common	
	WES	Coding regions	1) Much cheaper as compared to WGS 2) Can help devise personalized treatment strategies 3) Fast rate of analysis since it uses high-throughput screening	1) Cannot analyse mutations in non-coding regions 2) Differences in results depending on the type of capture method used 3) Missing of up to 3% of coding region mutations due to probe failure 4) Requires a large quantity of samples	Nakagawa et al., 2015
	WGS	Non-coding regions	1) Can help devise personalized treatment strategies 2) Fast rate of analysis since it uses high-throughput screening 3) Can analyze free circulating DNA and single-cell samples	1) Requires a high amount of investment 2) Still in the primitive stage and requires improvements to be made in sequencing technologies as well as informatics and computer resources 3) Informed consent-related ethical issues	Daniels et al., 2012; Nakagawa et al., 2015
Bio-chemical tests	ELISA	Proteins	1) Accurate 2) Sensitive 3) Specific 4) Reliable and Robust 5) Highly reproducible	1) Labour intensive. Requires skilled personnel to get accurate results and prevent any errors. 2) Tedious 3) Requires high sample volume	Hosseini et al., 2018
	RIA	Proteins	1) Sensitive 2) High specificity 3) Cost of running each sample is low	1) Very expensive for initial set-up 2) Has lower accuracy and precision	Landon and Moffat, 1976

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	Protein microarray	Proteins	1) Simple technique 2) Fast analysis due to the use of HTS 3) Multiple biomarkers can be analyzed simultaneously	1) High background signal 2) Chances of cross-reactivity 3) Low sensitivity and specificity	Chandra et al., 2011
	Western Blotting	Proteins	1) Highly sensitive 2) Straight-forward method 3) Simple to perform	1) Time-consuming 2) Lack of reproducibility 3) High error rate 4) Requires a large amount of sample 5) Multiple protein analysis is difficult	Furrer et al., 2015; Mishra et al., 2017

Genetic testing

Conventional approaches

Conventional genetic testing involves testing for genes inherited from one generation to another and may increase the risk of cancer if inherited. People who inherit these mutations do not necessarily suffer from cancer but are at a higher risk of getting cancer in their lifetime. Therefore, I generally recommend that people who have first-degree relatives who have cancer be associated with such mutations (Caswell-Jin et al., 2019; Nicolosi et al., 2019). Various techniques are utilized to identify the different types of inherited mutations through genetic testing. These approaches are explained along with its description in Table 3.

Allele-specific Polymerase Chain Reaction (ASPCR)

ASPCR is a technique commonly used to identify SNPs. It employs two inner primers to detect the SNPs, one complementary to the wild-type allele and the other to the mutant allele. The primer complementary to the wild-type allele is refractory to extension with the primer for the mutant allele and vice versa. A third type of primer, the outer primer, is used, complementary to both sequences and acts as a control. The products of ASPCR are then run on separate gels, one each for the wild-type allele and mutant allele and detected using autoradiography. The gels show three different kinds of products, one product, each corresponding to the specific inner primer used and the third product, which is a common product corresponding to the outer primer (Gaudet et al., 2009).

PCR and Sanger sequencing

Sanger sequencing is the most commonly used method to identify SNPs and small insertions or deletions in the gene. It utilizes four different reaction mixtures, each containing a DNA template whose sequence is to be detected, a primer, DNA polymerase and all the four types of deoxy nucleotide triphosphate (dNTPs), which include deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP). Each reaction mixture contains only a single type of dideoxy nucleotide triphosphate (ddNTPs), which aredideoxy adenosine triphosphate (ddATP), dideoxycytidine triphosphate (ddCTP), dideoxyguanosine triphosphate (ddGTP), dideoxy thymidine triphosphate (ddTTP) which are modified deoxynucleotides lacking a 3'-OH group. These ddNTPs are either fluorescently or radioactively labelled. Following the binding of the primer to the template strand, the DNA polymerase starts adding dNTPs complementary to the template strand causing the DNA strand to be extended. This strand extension stops following the addition of a ddNTP because of the lack of a 3'-OH group. There are four different types of strands formed in the four test tubes. Following PCR amplification, the contents of these test tubes are run in four different lanes on gel

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electrophoresis, with each lane corresponding to one nucleotide. The gel is visualized using autoradiography and read from bottom to top to get the sequence of the template strand. The difference in the sequence tells about the genetic change that has occurred (Katsanis and Katsanis, 2013).

PCR and pyro-sequencing

Pyro-sequencing technique is based on the principle of 'sequencing by synthesis' and detects the nucleotide added by DNA polymerase by detecting a visible light signal. The reaction involves the use of a single DNA strand whose sequence is to be determined. This DNA strand is hybridized into a sequencing primer. The reaction mixture consists of DNA polymerase, dNTPs, ATP sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate (APS) and luciferin. The reaction starts with the sequential addition of the solutions of dNTPs. When DNA polymerase adds a base that is complementary to the first base of the single-stranded DNA, it causes a release of the pyrophosphate (PPi) group, which is converted to ATP by ATP sulfurylase in the presence of APS. This ATP is a substrate for converting luciferin to oxyluciferin mediated by the enzyme luciferase. Oxyluciferin gives a visible light signal that is detected with the help of a camera (Marques et al., 2009). The enzyme apyrase then degrades the unincorporated dNTPs and ATP, following which the new cycle starts. This technique commonly detects SNPs in inherited genes (Ronaghi, 2003).

PCR and Primer Extension

Single-Base extension involves using ddNTPs that are fluorescently labelled using different fluorescent tags to detect the SNP. This technique utilizes a primer that is complementary to the sequence immediately upstream of the SNP. The DNA polymerase adds a single ddNTP complementary to the SNP and can be detected from the fluorescence observed following the addition of the ddNTP. The lack of 3'-OH group in the ddNTP prevents further addition of bases. The second approach utilizes fluorescently labelled dNTPs and an oligonucleotide primer. Suppose the primer has a sequence complementary to the specific allele. In that case, it hybridizes completely, allowing the DNA polymerase to add the next dNTP. In contrast, lack of complementarity, even at a single base, leads to a mismatch preventing the addition of the next dNTP (Ae et al., 2005). The fluorescent signal is then detected (Kim and Misra, 2007).

Molecular Beacons

Molecular beacon is a specially designed single-stranded probe of oligonucleotides consisting of a few complementary bases at both ends. This complementary sequence leads to the formation of a hairpin loop. One end of the probe is attached to a fluorophore, while the other end is attached to a quencher. The close proximity of the two prevents fluorescence. The probe is so designed that it is complementary to the sequence to be detected. When it encounters the complementary sequence, the hairpin loop opens up to form a straight structure that binds to it, separating the fluorophore and the quencher. This leads to the detection of fluorescence. On the other hand, when the probe encounters a sequence having an SNP, it does not hybridize and stays in its original hairpin conformation, thus exhibiting no fluorescence(Marras et al., 2003).

Multiplex ligation-dependent probe amplification (MLPA)

MLPA uses two oligonucleotide probes, one complementary to the region is directly upstream of the SNP that is to be detected, and the other is complementary to the region downstream of the SNP. The first primer provides the 3' end, while the second primer provides the 5' end for ligation. Only when the first primer is exactly complementary to the target DNA can the ligation of the two probes occur. If the probe encounters even a single nucleotide that is not complementary to it, the ligation reaction does not proceed due to a mismatch. The ligation products are amplified using multiplex PCR and detected using capillary electrophoresis (Homig-HolzelandSavola, 2012). Besides detecting SNPs, MLPA can also detect chromosomal abnormalities(Katsanis and Katsanis, 2013).

Fluorescent in-situ hybridization (FISH)

FISH is used to detect abnormalities in the chromosome, like changes in the chromosome copy number, amplification, deletion, translocation and duplication (Halling and Kipp, 2007). It utilizes a DNA probe that hybridizes to the complementary sequence on the chromosomal preparations fixed on unstained slides. The nucleotides may be directly labelled using a fluorescent marker or may be attached to reporter molecules which in turn may bind to fluorescently labelled antibodies and are visualized using fluorescent microscopy (Volpiand Bridger, 2008).

Novel approaches

Conventional technologies focused on direct genetic testing that involved testing individuals for variations in genes that led to a particular type of cancer and were only capable of testing for changes at a smaller level. With the advent of Next Generation Sequencing (Xia et al., 2006) technologies, there has been a paradigm shift in the focus

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of genetic tests towards indirect genetic testing allowing for the analysis of multiple genes simultaneously. Also, it involves a comparison of the DNA of affected and unaffected individuals to evaluate patterns of inheritance. Following are some of the applications of the NGS technology wherein they are used to detect changes in multiple genes, whole exomes and whole genomes (Katsanis and Katsanis, 2013).

Multigene panel testing

With the advances in sequencing technologies and the advent of NGS techniques, it has become possible to test multiple genes for inherited mutations using panel testing simultaneously. These panels include genes of high as well as moderate penetrance and are generally employed for detecting hereditary cancer syndromes that are associated with mutations in many genes and which show overlap with their associated phenotypes concerning the presentation and associated malignancies (Hall et al., 2014). Clinicians and patients prefer multi-gene testing as it is a rapid, cost-effective and time-saving alternative to single-gene testing. Also, multi-gene testing can help identify deleterious mutations in patients whose family history is unknown or in those where the family size is too small for analysis and where the pedigree cannot provide sufficient insights. Apart from this, panel tests can be used for those patients with atypical cancer phenotypes and where the family histories deviate from standard testing criteria. Though multi-gene testing has its advantages, the limitations must be considered while recommending them to patients. One of the most commonly encountered disadvantages involves variance of uncertain significance (VUS) caused due to the inclusion of a large number of genes in the panel, especially those having moderate penetrance, which leads to complexity in interpretation. Another disadvantage includes requiring skilled personnel to interpret the results and prevent patient confusion and anxiety (Hall et al., 2014; Kurian and Ford, 2015).

Genomic testing

Genome consists of the entire DNA content of the cell. Genomic analysis tests for somatic mutations that may develop during a person's lifetime instead of genetic testing, which detects inherited mutations. Also, since large structural variations like deletions and translocations are difficult to detect using just sequencing techniques, mapping the entire genome through genome testing can be of more value. Apart from just testing for cancers, genomic testing can also provide insights into the metastatic processes (Wise and Lawrence, 2019). The two most common types of genome testing utilized for analysis which are whole genome sequencing (WGS) and whole exome sequencing (WES), are discussed briefly.

Whole exome sequencing

WES involves sequencing and identification of mutations in the genome's protein coding regions and is the main platform for cancer genome sequencing. WES helps identify the pathways and mechanisms leading to cancer and new cancer-causing genes (Lee et al., 2022). Ning Yuan Lee et al. (2022) performed whole-exome sequencing of BRCA-negative breast cancer patients and identified 49 novel pathogenic variants in 37 genes associated with breast cancer predisposition (Rabbani et al., 2014).

Whole genome sequencing

Cancer occurs due to mutations not only in the coding regions but also in the non-coding regions. Very little information is available about the somatic mutations in the non-coding regions. Whole genome sequencing can not only help detect mutations in the un-translated regions, introns and regulatory elements but can also provide an idea of the role of these elements in the pathways underlying carcinogenesis. It can also help identify biomarkers for a particular type of cancer and aid in providing the patient with personalized medication. Also, WGS can be used to identify mutations in the oncogenes and tumour suppressor genes that behave as driver genes by providing a growth advantage to the cancer cells (Nakagawa et al., 2015).

Biochemical testing

Biochemical testing involves the detection of various biomarkers like hormones, enzymes, glycoproteins, oncofetal antigens or amines that are elevated because of underlying neoplastic conditions. They may be collected from urine or plasma, and the levels may be analysed using various immunoassays. Biochemical testing mainly focuses on analyzing the changes in the protein levels caused due to altered gene or chromosome expressions (Neville and Cooper, 1976). The currently analyzed biomarkers suffer from problems like low sensitivity and specificity. Choosing the correct biomarkers that are closely associated with the tumour may aid in the early detection of the tumour, help in prognosis and decide the therapeutic options (Kulasingam and Diamandis, 2008; Neville and Cooper, 1976). Following are some of the commonly employed techniques used for biochemical testing in cancer.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA uses wells on which the biomarker to be detected is coated. Following coating, the primary antibody (Ab) specific to the biomarker of interest is

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added to the well. The primary Ab is linked to an enzyme. Once the primary Ab binds to the biomarker, the substrate to the enzyme is added. The substrate gets converted to a coloured product whose intensity can be measured to quantify the biomarker present in the sample. A modification to this may involve the use of a secondary Ab bound to an enzyme. The enzyme-linked secondary Ab binds to the primary Ab and then gives a colour reaction following the addition of substrate. The wells are washed before adding the second Ab to remove any unbound Ab (Engvall, 1980). Apart from the direct ELISA, various modifications are also used to improve detection efficiency. They include sandwich ELISA that uses wells coated with a capture Ab. Competitive ELISA uses unlabelled Antigen (Ag)-Ab complexes and reverses ELISA that uses scavenger antigens to detect the biomarker (Aydin, 2015; Emmerich et al., 2006).

Radioimmunoassay (RIA)

This technique implements antigens that are radioactively labelled using tyrosine-attached isotopes of iodine, such as 125-I. This radiolabelled Ag is called the 'hot' Ag. These are then allowed to bind with a known amount of Abspecific to that Ag. Following binding, a sample is withdrawn from the patient containing the unlabelled 'cold' Ag and added to the radiolabelled Ag-Ab complex mixture. This 'cold' Ag competes with the 'hot' Ag for binding with the Ab. As the amount of 'cold' Ag is increased, more 'cold' Ag binds to the Ab displacing the 'hot' Ag from it. The radioactivity of the unbound Ag can be measured after the separation of the bound Ag (Waldmann and McIntire, 1974).

Protein microarrays

Protein microarrays are similar to ELISA but instead use microscope slides which are coated with nitrocellulose or gels to enhance protein binding. Protein microarrays may be of different types, including analytical microarrays, reverse-phase microarrays and functional microarrays, out of which the analytical microarrays are usually used to analyze the biomarkers. A capture agent, either an Ag or Ab, is coated on the solid array support and incubated with the test sample to be detected. It is then exposed to the specific labelled secondary Ab for detection (Chandra et al., 2011).

Western Blotting

Western blotting is a technique used to detect protein biomarkers by denaturing the proteins and separating them in their native form using gel electrophoresis. Sodium Dodecyl Sulphate (SDS) is used to denature the proteins and give them a uniform negative charge, ensuring they are separated based on their molecular weights. The proteins to be detected are then loaded and run on a gel made up of polyacrylamide for separation. The separated proteins are then transferred to a nitrocellulose membrane, which is visualized using the Ponceau stain. After visualization, the membrane is incubated with the primary Ab specific to the protein biomarker. Non-specific binding is prevented by pre-incubation of the membrane in a blocking solution like non-fat dry milk. The next step involves incubating the membrane with secondary Ab and visualization using autoradiography, chemiluminescence or densitometric scanning (Furrer et al., 2015; Li et al., 2011).

Artificial Intelligence (AI) in cancer testing

AI is a mimicking tool which simulates human intelligence in a non-living agent. With systematic data management, analysis and interpretation methodologies, AI is initiating a prototype shift towards healthcare (Dias and Torkamani, 2019).

Various diseases like cancer, cardiovascular diseases, neurological disease and genetic disorders have been explored using AI tools. However, diagnosing cancer at its initial stages and tracking its progression is still a challenge, with a lack of accuracy in identifying and analyzing results. Also, it is difficult to identify certain classes of cancer at early stages as they do not show any specific symptoms and signs on scans. Thus, working on a multi-variate diagnostic tool with a high-power resolution is essential to improve cancer prediction (Obermeyer and Emanuel, 2016). Therefore, AI can be explored in conjugation with various sophisticated mathematical models and machine learning tools to improve the accuracy of detecting, analyzing and diagnosing such critical disease conditions (Allahyar et al., 2019; Mitchell et al., 2017).

Breast cancer is one of the most aggressive types of cancer, with its complex clinical behaviour and morphological features making it difficult to detect and treat. Various AI tools have been developed to improve the overall detection and, thus, the prognosis of these multifactorial types of cancer. Sun et al. (2018) in China worked on Multimodal Deep Neural Network along with the fusion of Multi-dimensional Data (MDNNMD) with 1980 patients in the age group of 60-61years, which helped in increased accuracy of prediction data when compared with single dimension data and other methods of detection (Sun et al., 2018).

Lu et al. (2019) collected data from 82707 patients and proposed a dynamic genetic algorithm-based online gradient boosting (GAOGB) model, which helped in the real-time optimization of the data with an increase in overall effectiveness and prognosis with 28%

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improvement in the accuracy of the results (Lu et al., 2019).

National Cancer Institute (NCI) researchers are working on improving the primary screening of prostate and cervical cancer using AI (Kalayil Nisha and D'souza Shona, 2022). A senior investigator at NCI, Mark Schiffman, collected around 60,000 cervical images of women to analyze their chances of developing cervical pre-cancer over 18 years. The developed computer algorithm using AI tools helped monitor the abnormalities seen in the cervical images. It would predict the chances of developing cervical pre-cancer 6 to 7 years into the future with twice as much accuracy as the doctors' prediction (Anon, 2020).

In the clinical diagnosis of genetic disorders, AI tools have helped accurately interpret data using standard statistical tools, which are usually impractical and error-prone when carried out by humans. Some of the well-known tools include machine learning methods like classical support vector machines for structured data and natural language processing for unstructured data. These techniques have been explored to explain various elements in clinical genomic analysis, including genome annotation, variant classification, variant calling, and phenotype-to-genotype correlation (Dias and Torkamani, 2019).

AI methods have shifted drastically from reading the traditional genomic sequence to analyzing specific mutations in genomic sequences obtained from tumour images with improved accuracy (Chowdhury and Maitra, 2022). For example, scientists at NCI used the basics of deep learning to analyze the variation observed between two types of lung cancer, squamous cell carcinoma and adenocarcinoma, and its accurate prediction of frequently mutated genes from the captured images.

Blood-Based multi-cancer early detection (MCED) tests

Pan-cancer tests/ MCED tests have shown the potential to detect multiple types of cancers non-invasive and easier. Artificial Intelligence and Machine learning combined with assays of various analyses circulating in the blood. These tests offer simplicity and ease over the conventional tests available. Table 4 summarizes some advanced MCED tests.

Table 4. Blood-Based multicancer early detection (MCED) tests

Name of the test	Description	References
Cancer Seek	Circulating cell-free tumour DNA (ctDNA) is analysed by multiplex PCR. It allows for the detection of multiple mutations, while immunoassays are used to measure the amounts of protein biomarkers.	Cohen et al., 2018; Vogelstein and Kinzler, 2019
Galleri	Methylation sites of free DNA are studied for cfDNA methylation. The observed patterns are recognized with the help of AI. As a result, conclusions regarding the presence of neoplasm and primary cancer site can be drawn.	Beer, 2021
DELFI	This test evaluates patterns using machine learning techniques and is based on fragment comes, disorganised DNA packaging in cancer cells.	(Mathios et al., 2021; Victor and Stephen, 2019)

Thus, AI tools and advanced blood tests will result in drastic improvements in the prognosis and survival rate of cancer patients, which will overall enhance the outcome of clinical cancer testing, thereby solving the challenges of cancer prediction in the foreseeable future (Huang et al., 2020).

Conclusion

Researchers all around the globe are striving hard to identify genetic disruptions, alterations in the genome or exome and chromosomal instability which would help in studying the complications of cancer and designing effective treatments to make a breakthrough in cancer pathogenesis. There has been a noticeable breakthrough in our interpretation of various pathogenesis of gene

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associated-cancers. Recent studies have especially gathered information which is bridging the gap between genetic derangement and cancer in a broader aspect. There are advances in experimental designs and tools witnessing the recognition and characterization of peculiar normal cellular structures and mechanisms which are involved in maintaining chromosomal integrity. Researchers are constantly exploring various molecular methods to identify the potency of gene expressions, faulty genes and proteins and determine novel biomarkers. With the increasing number of cancer cases worldwide, there has been an enhanced need for testing the genetic changes, chromosomal instabilities and abnormal protein expression patterns in the disease. The advent of NGS technologies has revolutionized cancer testing. Though a lot is being done, there is still a long way to go before we can completely find a cure for cancer. With early diagnosis being the only way to achieve a complete cure, such an influential framework for cancer testing will help us save more lives and guide us in understanding the mechanisms involved in carcinogenesis and the targets to exploit in the treatment of cancer.

Conflict of interest

Nil

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